METABOLIC PATTERNS IN BIVALVES IX. SOME ASPECTS OF NITROGEN METABOLISM IN MARINE BIVALVES, WITH SPECIAL REFERENCE TO THE MUSSEL, MYTILUS EDULIS

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METABOLIC PATTERNS IN BIVALVES
IX. SOME ASPECTS OF NITROGEN METABOLISM IN MARINE BIVALVES, WITH SPECIAL REFERENCE TO THE MUSSEL, *MYTILUS EDULIS*

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(With eight tables)

INTRODUCTION

After finding out the localized distribution of guanine-deaminating activity in the gill lamellae of the clam, *Meretrix meretrix lusoria* (Gmelin), (Ishida 1954 a), the author has carried out several investigations in succession, concerning the catabolism of purines and their derivatives in the soft parts of certain pelecypods. Guanase was found active in the oyster, the marine mussel, and certain clams (Ishida 1956). The activity to dehydrogenate hypoxanthine was found very high in pedal, hepatic, and other tissues of the clam, *Meretrix* (Ishida 1954 c, 1955 c) and somewhat higher in certain tissues of the mussel, *Mytilus* (Ishida and Tsuzuki 1956). This dehydrogenation must be due to the action of xanthine oxidase which will transform xanthine to uric acid.

As is well known, uric acid is the main constituent of excreta in certain animals, especially those which are bound to terrestrial habitats. In such animals this substance with no doubt is synthesized through certain processes, by capturing amino groups derived from amino acids. It is also well known that there is another source from which uric acid is derived. It is from this latter source, the nucleic acid, that man and other primates excrete slight amounts of uric acid. This substance, however, will be decomposed further into allantoin in other mammalian groups, or into simpler compounds in many of the lower animals including certain molluscans.

According to Brunel (1938) the marine mussel, *Mytilus*, seems to be equipped with certain kinds of enzymes which decompose, step after step, purines and derivatives down to simpler substances and finally to carbon dioxide and ammonia. These last substances will be produced by the activity of urease which attacks

urea hydrolytically.

The present author has confirmed that this activity of decomposing urea is remarkable in the gills, mid-gut glands and other parts in the clam, *Meretrix* (Ishida 1954 b). On the other hand, the author and his coworker described a high activity of uricase in the mid-gut gland in the same clam and also in an allied species named *Venerupis philippinarum* (Ishida and Tsuzuki 1955).

On the basis of these findings the author has been believing that all of the marine bivalves will decompose purines to the very last products, carbon dioxide and ammonia, following the ordinary, well-known path from guanine through xanthine, uric acid, allantoin, allantoic acid and urea.

In another work (Ishida 1955 a) the author observed that *Corbicula japonica*, the species inhabiting estuarine, brackish waters, was much more active than *Corbicula leana*, the inhabitant of upper, freshwater streams, in decomposing urea to the simpler products. This result seemed very interesting to the author, for it looked as if to suggest certain relations between the activity of urease in the molluscans and the concentration of salts in their aquatic environment. It was also considered that this result may reinforce, in a certain way, the conclusion of Florkin that the freshwater bivalves, represented in his treatise by *Anodonta*, lack urease which is present in marine species, for instance in *Mytilus* as shown by Brunel (Brunel 1938, Florkin 1945).

Then the author investigated some more bivalves such as *Venerupis philippinarum*, *Anadara inflata*, and *Mactra sulcataria*, all inhabiting sea water. They showed considerable activities of urea-deamination in their gills, mid-gut glands, mantles and feet (Ishida 1955 b).

After having observed these bivalves the author was rather surprised when he found that the urea-deaminating acticity was practically absent from tissues of the mussel, *Mytilus edulis*, the very bivalve which Florkin has chosen as the representative of the marine lamellibranchs (Florkin 1945). First it was considered that certain environmental or physiologic conditions might be related with such an extreme scarcity of urease, but the author has failed to get positive proofs for this. Rather contrarily, most of his successive investigations showed little or no activity of urease in this mussel. Such results will be given presently in this paper.

It was also found that the oyster, *Ostrea gigas*, which was very active in deaminating guanine (Ishida 1956), was devoid of urease, as will also be shown presently.

Thus the author came to the opinion that certain bivalves, like the above two species, might lack urease, or might at least be very short of it, and that they might choose some other way of excreting nitrogen compounds of nucleic acid origin. Further he considers that there might be taking place certain processes

to synthesize uric acid in some bivalves. As will be reported elsewhere (Ishida and Tsuzuki 1956) the activity of uricase seems very slight in the mussel, whereas the xanthine oxidase was found quite active in the same animal. Some of the results shown in the following revealed a considerable amount of uric acid reserved or accumulated in certain parts of the mussel, and suggested the uricotelism in this animal.

This paper deals mainly with the urea-deaminating activity and the distribution of uric acid in some marine bivalves, especially in the mussel, *Mytilus edulis*.

Before giong further the author likes to express his heartiest thanks to Professor Shichiroku Nomura without whose encouragement this paper could not have been completed. Professors Saburo Isaka and Atsushi Fujita in the Chiba University have been very generous to help the author in many ways. They should be acknowledged here. Thanks are also due to Messrs Kiyoshi Tsuzuki and Hisayoshi Nagashima for their assistance.

PART 1. DEAMINATION OF UREA

As cited above, the urea-deaminating activity was very remarkable in some marine bivalves. The species investigated hitherto are *Meretrix meretrix lusoria* (Ishida 1954 b), *Venerupis philippinarum*, *Mactra sulcataria*, and *Anadara inflata* (Ishida 1955 b). It was also found that the brackish species, *Corbicula japonica*, and the freshwater inhabitant, *C. leana*, have the activity of urease (Ishida 1955 a).

In contrast with these the results of determinations in the mussel and oyster were negative in almost all cases, as will be shown in the following.

MATERIAL AND METHOD

The mussel, *Mytilus edulis*, was collected in the vicinity of the Misaki Marine Biological Station, Kanagawa Prefecture. Some of the specimens were kept for days or weeks in the laboratory or in an outdoor pool before use, while some were investigated immediately after being brought into the laboratory.

The oyster, *Ostrea gigas*, was obtained from a bed in Goi, Chiba Prefecture. The determinations were carried out with fresh specimens.

The total soft body, gill lamellae, mid-gut gland, mantle and foot were investigated, whilst the adult oyster lacks the last-named organ.

Each of these organs was weighed, ground in a mortar and extracted with neutral phosphate buffer, of which the volume was always fifteen times that of the tissues. In the case of total body, however, more highly concentrated extracts were used, as will be shown in each place.

The extract was centrifuged and 0.8 cc of the supernatant was placed in a Conway unit in which it was mixed with 0.2 cc of 0.1 per cent urea solution.

This substrate was replaced by water in the control preparation. After 15 minutes of standing at room temperature 1 cc of saturated potassium carbonate solution was added to the above mixture. This reagent will drive off ammonia which will be absorbed by 1.2 cc of $N/1000$ sulfuric acid in the central part of the unit. When the absorption of ammonia was completed about 1 hour after the addition of potassium carbonate, exactly 1 cc of the sulfuric acid was pipetted off into a test tube in which it was diluted with the addition of 4 cc of water. The tube was shaken well and 0.5 cc of Nessler reagent was added. The ammonia content was determined electrophotometrically, and was expressed as nitrogen in γ as produced by extract of 100 mg tissues. The procedure described above is exactly the same as that employed in former investigations in which the same concentration of tissue extract, the same amounts and concentrations of reagents, and the same length of time of reaction (15 minutes) gave the results remarkable enough to show high activities of urease in other lamellibranchs.

RESULTS

Experiment 1 (*Mytilus edulis*)

The specimens used in this experiment were collected on November 11, 1954, and were brought to the laboratory where they were placed in an outdoor pool of sea

Table 1.

Showing ammonia-nitrogen (N) in γ produced by extract of 100 mg tissues of gill, mid-gut gland, mantle, and foot of *Mytilus edulis*, immediately after dissection (0 min.) and 15 minutes (or 30 minutes) thereafter in the presence (exp) and absence (con) of excess urea. Photometric readings (-log T) are also shown. The specimens were kept in normal sea water in the pool for 8 days after collection, then in diluted sea water (Cl=4.9g/L) for 5 days. (Experiment 1).

Organ	Case	Con.						Exp.				
		0 min.		15 mins.		30 mins.		15 mins.		30 mins.		
		−log T	N	−log T	N	−log T	N	−log T	N	−log T	N	Difference exp - con
Gill	A	.014	2.2	.015	2.4			.015	2.4			0
	B	.005	0.8	.005	0.8			.005	0.8			0
	C	.023	3.6			.030	4.6			.025	4.0	(−0.6)
Mid-gut gland	A	.070	10.8	.080	12.4			.080	12.4			0
	B	.050	7.8	.056	8.6			.065	10.2			1.6
	C	.065	10.2			.070	10.8			.069	10.8	0
Mantle	A	.023	3.6	.022	3.4			.019	3.0			(−0.4)
	B	.006	1.0	.005	0.8			.007	1.0			(0.2)
	C	.021	3.2			.030	4.6			.030	4.6	0
Foot	A	.016	2.6	.026	4.0			.015	2.4			−1.6
	B	.013	2.0	.005	0.8			.012	1.8			1.0
	C	.025	4.0			.020	3.2			.023	3.6	(0.4)

Note: The figures in parentheses are within the range of inevitable errors.

water. A heavy rainfall diluted this water so much that the chlorine content decreased down to 4.9 g per liter. This happened on November 19. On November 23, *i.e.*, four days after the dilution of sea water, the specimens were dissected and gills, mid-gut glands, mantles and feet were investigated as regards their activity of urea-deamination. As is shown in Table 1 the activity in these organs appeared to be very low, being nil in most cases.

Except for mid-gut gland the production of ammonia by tissues, either in the presence or the absence of the substrate, was very low, so that the obtained values were less accurate especially when the photometric readings (−log T) were less than 0. 010.

Experiment 2. (*Mytilus edulis*)

As the activity of urea-deamination was not confirmed in the former experiment, and as this negative result might be due to the decreased salinity of the environmental water, some specimens were transferred from the pool into a jar in which sea water with a higher concentration (*ca.* 16 g Cl per liter) was contained, and after 20 or more days, these mussels were investigated. Some of the results in Table 2 may suggest that there seems to have occurred some slight deamination of urea in certain cases, but no positive proofs for this could be

Table 2.

Showing ammonia-nitrogen in γ produced in 15 minutes at room temperature by neutral extract of 100 mg tissues of gill, mid-gut gland, mantle and foot of *Mytilus edulis* in the presence (exp) and absence (con)of urea. Photometric readings (−log T) are also given. The specimens were kept in normal sea water in the pool for 8 days after collection, 5 days in diluted sea water (Cl=4.9 g/L) and then transferred into normal sea water in the laboratory and kept there for 20 days (A) or 21 days (B) or 27 days (C). (Experiment 2)

Organ	Case	Con.		Exp.		
		−log T	N	−log T	N	Difference, exp - con
Gill	A	.035	2.4	.040	3.2	0.8
	B	.059	7.6	.060	7.8	(0.2)
	C	.057	8.8	.056	8.8	0
Mid-gut gland	A	.111	14.2	.125	16.2	2.2
	B	.103	14.4	.108	15.2	0.8
	C	.125	19.4	.125	19.4	0
Mantle	A	.044	3.8	.050	4.8	1.0
	B	.074	10.0	.072	9.6	(−0.4)
	C	.049	7.6	.047	7.4	(−0.2)
Foot	A	.026	1.0	.038	3.0	2.0
	B	.066	8.6	.070	9.2	0.6
	C	.038	6.0	.037	5.8	(−0.2)

Note: The figures in parentheses are within the range of inevitable errors.

given in majority of them.

Experiment 3. (*Mytilus edulis*)

The determination experiments were repeated with the specimens of *Mytilus edulis* which were kept moist under the covering of sea weeds during the hours of transportation from seaside to the laboratory. Either in June (Cases A and B) or in October (Cases C and D) no effect of the presence of excess urea was observed (Table 3.)

Table 3.

Showing ammonia-nitrogen in γ produced in 15 minutes at room temperature by neutral extract of 100 mg tissues of gill, mid-gut gland, mantle, foot and total soft body of *Mytilus edulis* in the presence (exp) and absence (con) of urea. Photometric readings (−log T) are also given. The specimens were investigated immediately after transported to the laboratory under the moist covering of sea weeds. Determinations for Cases A and B were carried out in early June, for C and D in October. (Experiment 3)

Organ	Case	Con		Exp		Difference, exp - con
		−log T	N	−log T	N	
Gill	A	.010	1.6	.013	2.0	(0.4)
	B	.010	1.6	.010	1.6	0
	C	.035	2.2	.034	2.2	0
Mid-gut gland	A	.050	7.8	.050	7.8	0
	B	.050	7.8	.051	8.0	(0.2)
	C	.160	22.6	.159	22.4	(−0.2)
Mantle	C	.053	5.2	.053	5.2	0
Foot	C	.025	1.5	.025	1.5	0
Total soft body	D	.132	2.3	.132	2.3	0

Note: The figures in parentheses are within the range of inevitable errors. Due to instrumental disorders the activity of mantle and foot in Cases A and B could not be determined. Total soft body (7.3 g) was extracted with 7.3 cc of buffer solution.

It is very hard to prove absence of a certain enzyme in a certain animal, but it may be said at least that the mussel showed hardly any sign of urea-deaminating activity under the conditions which allowed other kinds of bivalves exert that activity very remarkably.

Experiment 4. (*Ostrea gigas*)

As shown in Table 4 the oyster, *Ostrea gigas*, did not show any sign of the activity of urea-deamination in any part of the soft body.

Table 4.

Showing ammonia-nitrogen in γ produced in 15 minutes at room temperature by neutral extract of 100 mg tissues of gill, mid-gut gland, mantle and total soft body of *Ostrea gigas* in the presence (exp) and absence (con) of urea. Photometric readings (−log T) are also given. (Experiment 4)

Organ	Case	Con		Exp		Difference, exp - con
		−log T	N	−log T	N	
Gill	A	.036	2.6	.037	2.8	(0.2)
	B	.040	3.2	.040	3.2	0
Mid-gut gland	A	.054	5.4	.052	5.2	(−0.2)
	B	.060	6.4	.062	6.8	(0.4)
Mantle	A	.036	2.4	.035	2.2	(−0.2)
	B	.044	3.8	.042	3.4	(−0.4)
Total soft body	C	.161	4.3	.159	4.2	(−0.1)
	D	.136	3.5	.136	3.5	0

Note: The figures in parentheses are within the range of inevitable errors. Total soft body was extracted with twice amount of buffer (2.5 g in 5.0 cc buffer in case C and 4.3 g in 8.6 cc in Case D).

PART 2. CONTENT OF URIC ACID IN VARIOUS PARTS IN THE MUSSEL AND CERTAIN OTHER BIVALVES

The absence of urea-deamination in the tissue extract of the mussel and the oyster suggested that certain kinds of bivalves might be quite different from others in their mode of nitrogen metabolism, or they might adopt more than two ways of catabolising nitrogen compounds. As will be shown elsewhere (Ishida and Tsuzuki 1956) the mussel and the oyster are less active than the clam and other eulamellibranchs in decomposing uric acid. It was also observed, by chance, that the uric acid content of the mid-gut gland extract of the mussel increased, though slight in amount, during the night of standing. It needs many more experiments to confirm the possible synthesis of uric acid in this mussel, but the above incidental observation led the author and his coworkers to the opinion that some attempts should be made to find out the fate of uric acid in the physiology of this animal. First, one of them scraped the inside surface of the shell valves and collected a little of white powder on which Folin's uric acid reagent was applied. The reaction was positive.

Then the author carried out a few preliminary tests and found that the byssus also gives a remarkable coloration in reaction to Folin's reagent. He observed, however, that the shell valves of other lamellibranchs are also positive in this reaction, though seemingly in a less degree than those of the mussel are.

In order to establish the characteristic occurrence of uric acid in the mussel the following experiments were carried out.

MATERIAL AND METHOD

Shell valves of *Mytilus edulis*, *Ostrea gigas*, *Anadara inflata*, *Mactra sulcataria*, *Meretrix meretrix lusoria*, and *Venerupis philippinarum* were investigated. Soft parts of *Mytilus edulis* and *Ostrea gigas*, as well as byssal tufts of the former species were also investigated. Specimens of *Mytilus edulis* were collected in the vicinity of Misaki Marine Biological Station and at Kurihama, Kanagawa Prefecture, those of *Ostrea gigas* were obtained from Goi, Chiba Prefecture, while all the other species were obtainable in Inage, Chiba Prefecture.

The shell valves were crashed and pulverized in a mortar and were extracted with 3.5*N* sulfuric acid for a few hours on a boiling water bath. The extract was filtered and was denatured with 20 per cent trichloroacetic acid solution. The byssal tufts and soft parts were treated in a similar way without being ground. After standing overnight the solution was filtered and 1cc of the supernatant was pipetted off into a 25 cc measuring flask in which it was mixed with 10 cc of urea-sodium cyanide solution and 2 cc of Folin's uric acid reagent. About 60 or more minutes after the addition of water up to the 25 cc mark the blue color was determined photometrically. The uric acid content was expressed in γ per 100 mg material.

RESULTS

Experiment 5.

Anadara inflata, *Mytilus edulis*, *Ostrea gigas*, *Mactra sulcataria*, *Meretrix meretrix lusoria* and *Venerupis philippinarum* were investigated.

In a conic flask 0.5 g of pulverized shell valve or byssal tuft was extracted

Table 5.

Uric acid content, γ per 100 mg, in pulverized shell valves of marine bivalves and in byssal tuft from *Mytilus edulis*. For details of experimental procedure see the text. (Experiment 5)

Species	Organ	Uric acid γ per 100 mg
Anadara inflata	shell valve	10.2
Mytilus edulis	〃	20.4
	〃	15.3
	byssal tuft	178.5
Ostrea gigas	shell valve	6.1
Mactra sulcataria	〃	7.7
Meretrix meretrix lusoria	〃	10.2
Venerupis philippinarum	〃	10.2
	〃	7.7

with 8 cc of 3.5 *N* sulfuric acid, for 1 hour on a boiling water bath. The extract was centrifuged. To 3 cc supernatant 1.5 cc of 20 per cent trichloroacetic acid solution was added. After standing overnight the solution was filtered off and the uric acid content was determined.

It is evident in Table 5 that mussel, *Mytilus edulis*, is exceedingly rich in uric acid. Very remarkable is that the byssal tuft contains uric acid about ten times that found in shell valves.

Experiment 6.

The mussel and the oyster were compared as regards the uric acid content in shell valves, byssal tufts and soft bodies. To 1 part of soft body 9 parts, to 1 part of shell valve 19 parts, and to 1 part of byssal tuft 199 parts respectively of 3.5 *N* sulfuric acid were applied. They were heated for 2 hours on a boiling water bath. The solution was filtered off and was denatured with the equal amount of 20 per cent trichloroacetic acid solution.

Table 6 shows the results.

Either in shell valves or in soft parts the mussel contains a definitely larger amount of uric acid than the oyster. The byssal tuft of the mussel showed an exceedingly high content of uric acid as compared with shell valves.

Table 6.

Uric acid content, γ per 100 mg, of shell valves and soft bodies of *Mytilus edulis* and *Ostrea gigas*. For details of experimental procedure see the text. (Experiment 6)

Species	Organ	Uric acid γ per 100 mg
Mytilus edulis	shell valve	32.0
	〃	30.0
	〃	38.8
	soft body	20.4
	〃	22.0
	〃	22.4
	byssal tuft	90.0
Ostrea gigas	shell valve	10.8
	〃	9.6
	〃	12.0
	soft body	17.8
	〃	13.2
	〃	13.2

Experiment 7.

In order to look into the distribution of uric acid among various parts of the mussel, gill lamellae, mid-gut gland tissues, foot, total soft body of which

byssal tuft was removed, byssal tuft, and shell valves were investigated. To 1 part of byssal tuft 49 parts of sulfuric acid (3.5 *N*) were added. For shell valves and soft organs 4 parts of the acid were added to 1 part of the material. The extract was denatured with the equal amount of 20 per cent trichloroacetic acid solution.

It was found that the content of uric acid in *Mytilus edulis* varies much with the part of the body. Very interesting is that the foot is as rich in uric acid as the byssal tuft. Deducing from the content in the total soft body the parts other than foot may not be very rich in uric acid. Among the parts investigated the gill lamellae were the poorest in uric acid content.

Table 7.

Uric acid content, γ per 100 mg, of shell valves, soft parts, and byssal tuft of *Mytilus edulis*. For details of experimental procedure see the text. (Experiment 7)

Organ	Urib acid γ per 100 mg
Gill	5.9 4.0
Mid-gut gland	10.0 6.9
Foot	70.0
Total soft body, without byssus	7.4
byssal tuft	69.0
Shell valve	16:3 10.0 16.4

In this experiment larger specimens were used. To get 2 g of branchial tissues, however, 4 individuals were needed ; for 2.5 g of mid-gut gland 3 individuals and for 0.9 g pedal tissues 6 individuals were needed.

Experiment 8.

To confirm the localized distribuaion of uric acid in foot and byssus these parts were investigated (1 part of material was extracted with 99 parts of 3.5 *N* sulfuric acid). The soft body, of which foot and byssal tuft were removed, were also investigated. (1 part extracted with 19 parts of acid). There seems to be considerable fluctuation in the uric acid content of these parts. Moreover, there might be considerable errors due to the method of extraction, for in most cases the volume or concentration of the acid was not sufficient to dissolve all the tissues and their products within limited hours of heating. In spite of all these, however, quite evident is that the foot and the byssus are definitely richer in

Table 8.

Uric acid content, γ per 100 mg, of foot, byssal tuft, soft body without foot and byssal tuft, and shell valves of *Mytilus edulis*. For details of experimental procedure see the text. (Experiment 8)

Organ	Uric acid γ per 100 mg	Remarks
Foot	126.0 80.0	0.1 g from 3 small individuals 0.2 g from 2 large individuals
Byssal tuft	116.0 132.0	0.2 g from 2 small individuals 0.2 g from a large individual
Soft body without foot and byssal tuft	14.0 13.2 19.0	smaller specimen smaller specimen larger specimen
Shell valve	16.0 17.2 9.6 10.4 14.8 14.8	a single valve weighed 6.5 g 〃 〃 5.6 g 〃 〃 4.9 g 〃 〃 5.8 g 〃 〃 1.6 g 〃 〃 1.4 g

uric acid than other parts (Table 8).

In this experiment two groups of specimens, larger and smaller, were used, but no relation between the uric acid content and the size or age of animal, as was suspected by comparing results of former two experinmets, was observed. The volume of sulfuric acid seems to be important to dissolve out completely the uric acid in the material. More exact investigations toward this point, however, will be carried out in future.

DISCUSSION AND CONCLUSION

From the results described in the first part of this paper it may be evident that the mussel, *Mytilus edulis*, and the oyster, *Ostrea gigas*, are quite short of urea-deaminating activity. Other bivalves, as the author has investigated in his previous works (Ishida 1954 b ; 1955 a, b), showed obvious activities in their tissue extracts to decompose urea under the conditions similar to those given to the present species, as investigated by the similar method. At least from the comparative point of view the mussel and the oyster may not be the animals which excrete ammonia in a copious amount. The author is not yet in the place to deny all the possibility of the occurrence of urease in these species, but he may say that there exist many other bivalved molluscs which are more adequate to be listed as examples of urea-decomposing species.

These two species, the mussel and the oyster, belong to different orders, but in respect of the activity of urea-deamination they are like each other. On the other hand, *Anadara inflata*, the member of Filibranchia to which *Mytilus edulis*

also belongs, showed a very high activity of urea-deamination as did eulamellibranchs such as *Mactra sulcataria*, *Meretrix meretrix lusoria*, and *Venerupis philippinarum*. No relation could be found between the occurrence of this activity and the taxonomic position of the animal.

One may postulate that the mussel and the oyster resemble each other in their ecologic characters, for both are sedentary and inhabit littoral, intertidal zones. In such a habitat the animals may sometimes be exposed to air and may have to endure a certain period of shortage of water. It may be advantageous under such circumstances to avoid production of free ammonia in the body which temporarily will be cut off from the surrounding aquatic world, and to keep catabolites in less soluble forms. However, no conclusive comment should be allowed without further investigations.

The author does not know anything about the fate of uric acid with precision, but, as cited above, *Mytilus edulis*, like *Ostrea gigas* and *Anadara inflata*, is less endowed with the activity to decompose uric acid, and this suggests to the author that most part of the purines in this animal may cease to be broken down by the stage of uric acid. On the other hand, an incidental observation suggested a possible increase of uric acid in the mid-gut gland of this species. If really this occurs in the living body, and if really the decomposition of uric acid does not take place in a noticeable degree, this substance must be rejected as such either in the form of urine or taking the form of *Speicherniere*.

The results of experiments described in the second part of this paper seem to show that *Mytilus edulis* has an evident tendency to accumulate uric acid in various parts of its body, especially in a very remarkable degree in the foot and byssus, in a less degree in shell valves, and in a lesser degree in other soft parts.

Whether does this substance exist in free form or as urate is not investigated yet. The high content of uric acid in the byssus may suggest a certain significant role played by this substance in making up that strong, elastic thread. The foot, which is in close relation in function with the secretion of byssal substances, may act as the reservoir of uric acid. Of course many detailed observations are needed before establishing any concrete ideas in this connection.

According to Kato and coworker uric acid and guanine are found as crystals in the connective tissues around the crystalline style sacs of certain gastropods (Kato and Kubomura 1955). They also suggest the occurrence of similar crystals in the similar tissue of lamellibranchs. Further they assume that uric acid crystals, which are formed and accumulated inside the connective tissue cells, will be discharged into intercellular spaces where they will be converted, by the action of unknown enzymes, presumably through xanthine, into guanine. Now, accordnig to these workers, the crystals of guanine are discharged into the crystalline style sac and then transported by the ciliary action into the alimentary tract.

According to these workers, therefore, guanine is assumed to be synthesized from uric acid in the molluscan tissues.

The present author does not know how could such a process be possible, but he is much interested in that the crystals of urate have actually been observed by them either in gastropod or lamellibranch tissues.

It is not seldom that animals accumulate uric acid somewhere within or outside their bodies. Either marine or land snail is known to excrete this substance. In lamellibranchs, however, there seems to have been no report except the one by Kato and coworker to give evidence to the excretion of uric acid out of the cells.

On the basis of the results of observations as described in this paper the present author assumes that certain kinds of bivalved molluscs are short of enzymes which catabolize purines to the last possible products, and instead, as he assumes, some of these animals may form, either starting from nucleic acid or from amino acids, purine compounds. These substances, as he further assumes, may be accumulated by certain kinds of bivalves in certain parts of their bodies, sometimes as important constituents in structures of high utility. *Mytilus edulis*, as the author assumes, may make up uric acid from certain source or sources, may use it as a constituent of byssus and shell valves, and may store it mainly in the tissues of foot which functions in coordination with the byssal secretion. Shell valves of this mollusc may also be assumed as an important depot of uric acid, for they contain this substance in a higher concentration than do the valves of other species.

Only a few experiments have been carried out as regards the uric acid content in the oyster. So far investigated, this animal does not seem to have any part in which uric acid is accumulated. The lack of the activity of urea-deamination, as well as the low activity of uricase, calls for further studies to elucidate the fate of uric acid in this shellfish.

Other kinds of bivalves should also be investigated in this regard.

SUMMARY

1. Deamination of urea by tissue extracts of various organs in the mussel, *Mytilus edulis*, and the oyster, *Ostrea gigas*, was investigated.

2. No positive result to prove the activity of urea-deamination could be obtained in either species.

3. Uric acid content of shell valves and other parts was determined in some bivalves.

4. The shell valves of *Mytilus edulis* showed the highest concentration of uric acid above those of others such as *Anadara inflata*, *Meretrix meretrix lusoria*, *Venerupis philippinarum*, *Mactra sulcataria*, and *Ostrea gigas*.

5. The soft body of *Mytilus edulis* contained more uric acid than that of *Ostrea gigas*.

6. Among various parts of *Mytilus edulis* the byssus and foot showed the highest and extraordinary content of uric acid.

7. Excretion in molluscs was discussed and the possible uricotelism in lamellibranchs was assumed.

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